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Edwards, K., Elliott, B. and Silva, C.

This is an accepted manuscript of an article published by Taylor & Francis in Journal of Sports Sciences, DOI: 10.1080/02640414.2019.1702269.

The final definitive version is available online:

<https://dx.doi.org/10.1080/02640414.2019.1702269>

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1 **Carbohydrate intake and ketosis in self-sufficient multi-stage ultramarathon**
2 **runners**

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4 Kate H. Edwards^{a, b*}, Bradley T. Elliott^a, Cecilia M. Kitic^b

5
6 a. Translational Physiology Research Group, School of Life Sciences, University of
7 Westminster, London, UK

8 b. Sports Performance Optimisation Research Team, School of Health Sciences, University
9 of Tasmania, Australia

10

11 *Corresponding Author:

12 Kate H Edwards: University of Tasmania, Sport Performance Optimisation Research Team,
13 School of Health Sciences, Locked Bag 1322, Launceston, Tasmania, Australia

14 Email: kate.edwards0@utas.edu.au Telephone: +61 3 6324 3999

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17 Abstract Word Count: 198

18 Main Body Word Count: 5017

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29 **runners**

30

31 **Abstract**

32 Ultra-endurance athletes accumulate an energy deficit throughout their events and those
33 competing in self-sufficient multi-stage races are particularly vulnerable due to load carriage
34 considerations. Whilst urinary ketones have previously been noted in ultra-endurance exercise
35 and attributed to insufficient carbohydrate (CHO) availability, not all studies have reported
36 concomitant CHO intake. Our aim was to determine changes in blood glucose and β -
37 hydroxybutyrate concentrations over five days (240 km) of a self-sufficient multi-stage
38 ultramarathon in combination with quantification of energy and macronutrient intakes, estimated
39 energy expenditure and evaluation of energy balance. Thirteen runners (8 male, 5 female, mean
40 age 40 ± 8 years) participated in the study. Glucose and β -hydroxybutyrate were measured every
41 day immediately post-running, and food diaries completed daily. CHO intakes of 301 ± 106
42 $\text{g}\cdot\text{day}^{-1}$ ($4.3 \pm 1.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) were not sufficient to avoid ketosis (5-day mean β -
43 hydroxybutyrate: $1.1 \pm 0.6 \text{ mmol}\cdot\text{L}^{-1}$). Furthermore, ketosis was not attenuated even when CHO
44 intake was high ($9 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$). This suggests that competing in a state of ketosis may be
45 inevitable during multi-stage events where load reduction is prioritised over energy provisions.
46 Attenuating negative impacts associated with such a metabolic shift in athletes unaccustomed to
47 CHO and energy restriction requires further exploration.

48

49 **Key words:** ketones, running, ultra-endurance, carbohydrate, performance, nutrition, energy
50 deficit

51

52 **Introduction**

53

54 Self-sufficient multi-stage ultramarathons are conducted over multiple days and athletes must
55 carry all necessary clothing, equipment, and food required for the race in a backpack. Extreme
56 environments, rough sleeping conditions and increased load carriage from backpacks result in
57 long days of high physical, mental and emotional effort. It is recognised that many participants
58 in ultra-endurance races compete in a state of negative energy balance and insufficient
59 carbohydrate intake (Costa et al. 2013; Wardenaar et al. 2015). The reasons have been well
60 explored elsewhere (Costa et al. 2016; Costa et al. 2017; Stuempfle and Hoffman 2015;
61 Stuempfle et al. 2013) but some of the most common are gastrointestinal issues and the inability
62 to consume enough calories to offset energy. In the case of self-sufficient events, these factors
63 are compounded by a deliberate decision by athletes to compromise energy intake for reduced
64 load to carry (Alcock et al. 2018; Lucas et al. 2016; McCubbin et al. 2016).

65

66 During periods of acute carbohydrate (CHO) insufficiency ketone bodies (acetoacetate (AcAc)
67 and beta-hydroxybutyrate (β -HB)) are produced as an integral component of homeostasis (Cox
68 and Clarke 2014). As blood glucose and insulin levels drop, free fatty acids are liberated from
69 adipose tissue and partially oxidised in the liver producing ketones (Cahill 1981). The brain can
70 then utilise these circulating ketone bodies for up to 60% of its energy requirements, the
71 remainder coming from gluconeogenesis (Cox and Clarke 2014; Egan and D'Agostino 2016).
72 Ketones also become an alternate energy source for the heart and skeletal muscle (Cox and
73 Clarke 2014; Egan and D'Agostino 2016) reducing nitrogen depletion and allowing for the
74 retention of lean muscle mass (Cahill 2006). This suggests that during a self-sufficient, multi-
75 stage ultramarathon when limited by restricted exogenous energy and CHO sources, being in a
76 state of ketosis may be beneficial to athletes.

77

78 In practice however, being in a state of ketosis during athletic endeavours is generally regarded
79 as undesirable due to the initial ‘adaptation period’ characterised by lethargy and fatigue, as well
80 as the potential for impaired performance due to glycogen depletion, inefficient utilization of
81 muscle substrates, and reduced exercise economy (Burke et al. 2017; Phinney et al. 1983; Yeo
82 et al. 2011). Although metabolic adaptations to low carbohydrate or calorie restricted diets
83 demonstrably occur within five days (Goedecke et al. 1999) it has been suggested that athletes
84 may require several weeks, if not months, to adapt fully (Volek et al. 2016). This may have
85 negative implications for athletes who experience this transition period during multi-stage ultra-
86 endurance events.

87

88 Early studies on starvation (Azar and Bloom 1963; Bloom and Azar 1963; Cahill 1981;
89 Consolazio et al. 1968) demonstrated the link between CHO insufficiency and ketosis.
90 Consolazio et al. (1968) similarly noted that when in a daily energy deficit of 11.7 MJ (2 800
91 kcal) induced through fasting and exercise, ketosis could be avoided by ingesting 1.8 MJ (420
92 kcal, ~100 g) of CHO per day, an amount equal to the carbohydrate requirements of the brain
93 (Cahill 1981). Subsequent guidelines have thus recommended a daily CHO intake of >100 g
94 combined with a fat intake of less than 160 g to prevent ketosis during periods of caloric
95 restriction (Marriott 1995; Montain and Young 2003). There is scarce data on ketosis and CHO
96 intake during ultra-endurance exercise. Although the presence of urinary ketones has been
97 reported in athletes during ultra-endurance events and attributed to insufficient CHO (Costa et
98 al. 2014; Costa et al. 2013), not all studies have reported concomitant CHO intake (Jablan et al.
99 2017; Weibel and Glonek 2007).

100

101 .

102

103 There is a growing body of nutrition research in the field of ultra-endurance sports, but the focus
104 is often on races where participants have access to exogenous food supplies through aid stations
105 and/or crew assistance. Nutritional intake during a self-sufficient multi-stage race is restricted to
106 what the participant is prepared to carry from the first day. This is, to our knowledge, the first
107 study to quantify energy, CHO intake and β -HB during a fully self-sufficient multi-stage
108 ultramarathon. The aim of this study was to determine changes in blood β -HB concentration
109 during five days of a self-supported multi-day ultramarathon in combination with quantification
110 of energy and macronutrient intakes.

111

112 **Methods**

113

114 *Ethics statement*

115 Ethical approval was granted by the University of Westminster FST Research Ethics Committee
116 (Application VRE1516-0780). All work was performed in accordance with the principles of the
117 Declaration of Helsinki and participants gave written, informed consent.

118

119 *Participants*

120 Participants were recruited from the pool of registered competitors via an email sent out by the
121 race organisers. Details of the study were also posted on a social media platform with a request
122 for volunteers.

123

124 *Race conditions*

125 The study was conducted during a 7-day self-sufficient, multi-stage ultramarathon in the
126 Namibian Desert in May 2016. Race organisers provided shelter for sleeping (10 person canvas

127 tents) and plain water at overnight campsites and plain water only at aid stations positioned
128 approximately 10 km apart on the course. Competitors were required to carry all other personal
129 and mandatory equipment, including food, in a backpack for the duration of the race. Race
130 regulations stipulated a minimum food requirement of 14 000 kcal for the entire race.

131
132 Course terrain was predominantly sandy (beach, dunes) with some vehicular dirt track, rocky
133 sections and salt pans (hard packed mud and coral-like terrain). Recorded temperatures ranged
134 between 16°C at night and 35°C during the day (mean daytime temperature 27°C ± 4°C).
135 Humidity ranged from 25% to 51%.

136
137 The competitors took seven days to complete the race, which totalled 250 km. This investigation
138 took place during the first five days of the race during which the participants completed a total
139 of 240 km. Each day commenced at 08:00 and stage distances for the first four days were: 38
140 km, 42 km, 42 km and 41 km. The fifth day, known as ‘the long stage’, was 77 km and
141 competitors were allowed 27 hours to complete the distance. This format is characteristic of this
142 series of races, and in practice results in most competitors having a full ‘rest’ day on day six
143 which is when the final measures were taken. Upon completion of the 10 km stage on day 7
144 (which was not included in the study) food and drink were provided by race organisers and then
145 participants had a two hour bus ride back to the host town

146

147 ***Study design***

148 Baseline measures were taken one day prior to the race (pre-race) and on the ‘rest day’ (day six),
149 a minimum of 12 hours following the finish of the long stage. Blood and food diary data was
150 collected on days one to five. A schematic of the study protocol is provided in Figure 1.

151

152 *** Figure 1 about here ****

153

154 *Performance*

155 Although this study was not interventional, data collection took place during a competitive race
156 event. Therefore, finishing times were converted into average velocity ($\text{km}\cdot\text{hr}^{-1}$) and used as a
157 measure of performance. Race timings were recorded via timing chips carried by competitors
158 and official results were provided by the race organisers.

159

160 *Anthropometry*

161 Anthropometric measures were taken with participants in their racing clothes comprised of shorts
162 (males) and shorts and bra tops (females) for both pre- and rest day measures. All participants
163 were sockless and shoeless during the measures. Height was measured pre-race to the nearest 0.1
164 cm (Seca 213 stadiometer, Seca, Birmingham, UK). Body mass was measured pre-race and on
165 the rest day to the nearest 100 g (Seca 877 flat scales, Seca, Birmingham, UK). Scales were
166 placed on a wooden board to provide a stable surface in the field.

167

168 The sum of skinfolds was determined pre-race and on the rest day using the four-site
169 Durnin/Womersley skinfold method (Durnin and Womersley 1974). Skinfold thicknesses were
170 measured on the right side of the body to the nearest 0.2 mm using Harpenden skinfold callipers
171 (Baty International, West Sussex, UK). All anthropometric measures were conducted by the
172 same investigator (technical error of measurement (TEM) of 3.5%)

173

174 *Glucose and β -hydroxybutyrate*

175 Every day, immediately post-stage, blood glucose (GLUC) and β -hydroxybutyrate (KET) were
176 measured via capillary sampling from the fingertip using two CardioChek analysers (Polymer

177 Technology Systems, Indianapolis, USA) and PTS Panels single-analyte test strip. Each analyser
178 was specific either to GLUC or KET throughout the study. Limits of detection for GLUC and
179 KET were 1.11-33.3 mmol·L⁻¹ and 0.19 - 6.72 mmol·L⁻¹ respectively. Analyser testing using
180 check strips was performed daily on both analysers.

181
182 Blood samples were collected with participants in a standardised seated posture, immediately on
183 crossing the finish line of the stage. Ketosis was defined as a blood β-hydroxybutyrate
184 concentration of ≥ 0.5 mmol·L⁻¹ (Volek et al. 2015).

185
186 Whilst the assessment of urinary ketones is a convenient and cost effective method in the field,
187 hyper- and hypohydration, both common issues in ultra-endurance events (Hoffman and
188 Stuempfle 2014; Hoffman et al. 2012), can result in false negatives and false positives
189 respectively (Brewster et al. 2017). Urine strip testing is subjective, semi-quantitative and cannot
190 control for how long urine has been sitting in the bladder. Blood analysis of ketones however
191 provides a quantifiable indication of current metabolic state through circulating β-HB.

192
193 ***Pack weights***
194 All competitors in the race had their packs weighed during check-in and results were provided
195 to the investigators by the race organisers.

196
197 ***Food diary and energy intake***
198 Participant food intake was restricted to what they chose to carry on day one, therefore similar
199 to the method employed in Stuempfle et al. (2013), individualised food diaries itemising every
200 food product carried on day one were prepared for each participant. The diary was provided to
201 participants at the end of each day for them to identify what and how much they had eaten, as

202 well as noting if food had been lost, thrown away or exchanged with/obtained from, another
203 competitor. Food diaries were then collected by the researcher following the last meal of the day
204 prior to the participant retiring for the evening. Analysis of the energy content and macronutrient
205 profile of foods was performed using Nutritics® dietary analysis software (v1.8, Nutritics Ltd,
206 Dublin, Ireland). All packaged foods were analysed according to manufacturer provided data.
207 Non-packaged foods were entered using equivalent foods existing in the database.
208 Approximately 1 week postrace, participants were sent an email with their nutrition data and
209 asked for clarifications and corrections.

210

211 *Estimated energy expenditure*

212 To provide a conservative estimate of total daily energy expenditure so as not to overestimate
213 differences in energy balance, three components were calculated for each participant for each
214 day of the study.

215

- 216 1. Sleeping: Predictive equations were used to estimate basal metabolic rate (BMR). The
217 Cunningham (Cunningham 1980) and Harris Benedict (Harris and Benedict 1918)
218 equations are recognised as being appropriate for athletic populations (Thomas et al.
219 2016), with the former more suitable for females and the latter for males (Jagim et al.,
220 2017). It was assumed that participants slept for 8 hours per 24-hour period.
- 221 2. Racing: Metabolic Equivalent of Task (METs) (Ainsworth et al. 2011) were used to
222 calculate the energy expenditure during racing each day based on average moving speed.
223 Participant weight for day one was defined as pre-race weight plus starting backpack.
224 Weight for subsequent days was calculated as pre-race weight plus starting pack weight
225 minus average daily food weight (food eaten the previous day).

226 3. Rest: The remaining time (24 hours minus sleep and racing time) was defined as ‘rest’
227 and calculated at 1.3 METs (reclining, talking) (Ainsworth et al. 2011).

228
229 Two competitors took ~20 hours to complete the ‘long stage’ (day 5). In this instance, the
230 assumptions were 20 hours racing, 1 hour ‘rest’ post-racing and 3 hours sleep for the 24 hour
231 period. All other participants took less than 15 hours and were therefore estimated to have 8
232 hours of sleep and at least one hour of rest on this day.

233
234 Although the thermal effect of food accounts for approximately 10% of total daily energy
235 expenditure, various factors such as body composition, macronutrient profile, meal timing,
236 exercise and stress can all influence the metabolic response to feeding (Secor 2009). Therefore,
237 rather than adding a blanket 10% to all estimates of energy expenditure this component has been
238 excluded. The authors recognise this may result in underestimated energy expenditure and
239 resultant calculated deficits.

240

241 ***Statistical analysis***

242 Statistical analysis was completed using GraphPad Prism 7.4 for Windows (GraphPad Software,
243 La Jolla California USA). All data was tested for normality using Shapiro-Wilk normality tests.
244 Energy intake on day 5 did not pass the test for normality. Repeated measures ANOVA with
245 Tukey’s post-hoc analysis was used to determine differences in variables between stages (energy
246 intake and blood measures). Pre-post measures were analysed using paired t-tests and Cohen’s d
247 was calculated for effect size.

248

249 Relationships between variables were determined using Pearson’s correlation coefficient. In the
250 case of non-parametric data (energy intake on day 5) Spearman’s rank coefficient was utilised.

251 Relationship strength was classified for the absolute r-value using thresholds of 0.1, 0.3 and 0.5
252 for small, moderate and large respectively (Hopkins et al. 2009). Significance was set at $p < 0.05$.
253 Data are presented as mean \pm standard deviation (SD).

254

255 **Results**

256

257 Seventeen participants from a field of 219 entrants volunteered for the study, of which 13 were
258 included in the final analysis (Table 1). Two withdrew from the race for reasons unrelated to the
259 study (injuries sustained while running), one participant elected to withdraw from the study but
260 continued with the race, and the nutritional data collected from one participant was incomplete
261 and as such, their data was excluded from analysis. The 13 remaining participants represented
262 6.6% of the finishing field: eight males (5% of male finishers) and five females (11% of female
263 finishers), All participants had trained for the event, had previous ultramarathon experience and
264 none reported cardiovascular or metabolic disorders..

265

266 *** Table 1 about here ***

267

268 *Estimated energy expenditure and energy and macronutrient intakes*

269 Average total estimated energy expenditure for five days of racing was 113.6 ± 23.6 MJ which
270 equates to 22.7 ± 6.6 MJ \cdot day⁻¹ and 2.3 ± 0.9 MJ \cdot hour⁻¹ during the racing periods of the day.

271

272 Compared to the race rules that stipulated competitors must start the race carrying food providing
273 a minimum of 14 000 kcal (58.6 MJ), participants carried an average of 63.6 ± 10.5 MJ on day
274 one (10.6 ± 1.7 MJ \cdot day⁻¹). Data from two participants were considered as outliers (>2 SD from

275 the mean) with one participant carrying 39.8 MJ and one carrying 88.5 MJ). The mean total
276 energy content of the food carried by the 11 other participants was 63.5 ± 3.6 MJ.

277
278 Mean energy intake over five days of racing was 48.0 ± 10.0 MJ (9.6 ± 2.6 MJ·day⁻¹, range: 4.75
279 -11.5 MJ). All participants were in negative energy balance of 64.6 ± 22.2 MJ (12.9 ± 6.3
280 MJ·day⁻¹) after five days (range: -44.6 to -2.7 MJ). Over the course of the race, no participant
281 consumed all the food they carried from the first day.

282
283 Participants consumed 301 ± 106 g·day⁻¹ CHO, contributing to 53% of total energy intake. Mean
284 fat (FAT) intake was 85 ± 33 g·day⁻¹ and protein (PRO) 85 ± 35 g·day⁻¹ representing 32% and
285 15% of total energy intake respectively. Corrected for body mass, participants consumed $4.3 \pm$
286 1.8 g·kg⁻¹·day⁻¹ CHO (range 1.6 to 9.1 g·kg⁻¹·day⁻¹), 1.2 ± 0.5 g·kg⁻¹·day⁻¹ FAT (range: 0.6 to
287 2.0 g·kg⁻¹·day⁻¹), and 1.2 ± 0.6 g·kg⁻¹·day⁻¹ PRO (range: 0.4 to 2.6 g·kg⁻¹·day⁻¹). Neither absolute
288 nor corrected intakes of energy (Figure 2A) nor macronutrients (Figures 2B, 2C and 2D) differed
289 between stages ($p > 0.5$).

290

291

292 *** Figure 2 about here ***

293

294 ***Blood glucose and β -hydroxybutyrate***

295 Mean GLUC measured at the end of each stage of the race was 4.8 ± 1.0 mmol·L⁻¹. Mean daily
296 concentrations are presented in Figure 3. Blood glucose did not differ between baseline ($5.1 \pm$
297 0.97 mmol·L⁻¹) and race days ($p > 0.2$). GLUC indicating hypoglycaemia (< 3.2 mmol·L⁻¹
298 (Mitrakou et al. 1991)) was reported once, in one participant on day five.

299 No participant registered blood β -HB $> 0.5 \text{ mmol}\cdot\text{L}^{-1}$ pre-race. Ketosis was observed in four
300 participants on day one and all participants on days two, three and five. There was a significant
301 decrease in KET concentration on day four compared to days three and five in all participants
302 ($p < 0.001$). On day four, three participants had KET $< 0.5 \text{ mmol}\cdot\text{L}^{-1}$. However, mean
303 concentrations remained significantly higher than baseline ($p < 0.002$). Three participants (all
304 different to those three with low KET on day four) did not meet the criteria for ketosis on the
305 rest day at least 12 hours following the finish of the long stage. Mean KET during the race was
306 $1.1 \pm 0.6 \text{ mmol}\cdot\text{L}^{-1}$.

307 *** Figure 3 about here ***

308

309 *Performance*

310 Participants took 39.9 ± 7.1 hours to complete the 240 km run during the study period at an
311 average velocity of $6.35 \pm 1.0 \text{ km}\cdot\text{hr}^{-1}$ including all stops at checkpoints along the course.

312

313 *Pack weights*

314 Mean pack weight for the full field of competitors that finished was $9.6 \pm 1.9 \text{ kg}$ (range 5.6 – 19
315 kg). Pack weight of the top 10 finishers was significantly lighter than those that finished in places
316 11 – 196 ($7.66 \pm 1.2 \text{ kg}$ vs $9.7 \pm 1.9 \text{ kg}$, $p = 0.006$).

317

318 *Correlations*

319

320 Neither absolute nor relative intakes of CHO or FAT correlated with KET (CHO: absolute $r =$
321 0.03 , $p = 0.9$, relative $r = -0.004$, $p = 0.99$; FAT: absolute $r = -0.14$, $p = 0.65$, relative $r = -0.20$,
322 $p = 0.50$). There was a strong, negative relationship between both absolute PRO intake and KET
323 ($r = -0.61$, $p = 0.03$) and relative PRO intake and KET ($r = -0.54$, $p = 0.056$).

324
325 Whilst there were no significant relationships between either daily energy intake or deficit
326 (MJ·day⁻¹) and GLUC (intake $r = -0.19$ $p = 0.53$, deficit $r = -0.12$ $p = 0.71$) or KET (intake $r = -$
327 0.28 , $p = 0.35$, deficit $r = -0.27$, $p = 0.36$), there was a moderate relationship between the
328 cumulative energy deficit and KET ($r = -0.44$, $p = 0.0002$).

329
330 Performance was not correlated with mean KET ($r = 0.22$, $p = 0.47$) but had a strong relationship
331 with total CHO intake ($r = 0.62$, $p = 0.02$).

332
333 As the first significant increase in KET occurred on day two, a Pearson correlation analysis was
334 used to assess the relationship between overall performance and the magnitude of β -HB
335 concentration increase from baseline to day two. There was a large positive relationship between
336 the magnitude of β -HB increase and overall performance that approached significance ($r = 0.54$,
337 $p = 0.06$, figure 4).

338
339 *** Figure 4 about here ***

340
341 **Discussion**
342
343 To the best of our knowledge this is the first study to investigate blood glucose and β -
344 hydroxybutyrate (β -HB) concentrations throughout a fully self-sufficient multi-stage
345 ultramarathon and the influence of nutritional intake on these substrates. The main finding is that
346 all participants in this study entered a state of ketosis within two days of the race commencing,
347 with ketosis not correlated with CHO intake.

348

349 Historically, an energy deficit *per se* has not been considered enough to induce ketosis
350 (Consolazio et al. 1968): a concomitant reduction in CHO availability to less than 100g must
351 occur. Here we show that despite a similar caloric deficit as that induced by Consolazio et al.
352 (1968) ketosis still occurred in all participants despite a mean intake of $301 \pm 106 \text{ g}\cdot\text{day}^{-1}$.
353 Furthermore, ketosis was still evident in participants who consumed up to $600 \text{ g}\cdot\text{day}^{-1}$ of CHO.
354 This lack of relationship between macronutrient intake and ketosis but large relationship
355 between cumulative energy deficit and ketosis suggests that the magnitude of the ongoing energy
356 deficit and the manner in which it is induced (i.e. exercise induced vs energy restriction) may
357 play a greater role than previously appreciated.

358

359 Prior studies have also noted the presence of urinary ketones in ultra-endurance athletes despite
360 apparently adequate CHO intakes (Costa et al. 2014; Costa et al. 2013; Jablan et al. 2017; Weibel
361 and Glonek 2007). Costa et al. (2013) noted that 46% of runners in a multi-day ultramarathon (5
362 days, 225 km) presented with urinary ketones indicative of ketosis at least once during the race
363 despite CHO intakes of $520 \text{ g}\cdot\text{day}^{-1}$ ($7.5 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$). Likewise, in a 24 hour ultramarathon,
364 urinary ketones were present in 90% of runners whose average CHO intake was 881 g ($37 \text{ g}\cdot\text{hr}^{-1}$)
365 (Costa et al. 2014). Weibel and Glonek (2007) found that in a six day race, 22 of 31 study
366 participants produced urinary ketones, and although dietary intake was not recorded, they
367 observed that some participants produced urinary ketones despite apparent high CHO intake.
368 Likewise Jablan et al. (2017) reported a significant increase in urinary ketones in 81% of
369 participants following a mountain ultra-marathon (mean race time 8.40 ± 1.28 hours) although
370 CHO intake was not quantified. All these studies have attributed the presence of urinary ketones
371 to insufficient CHO intake, but have not further explored the physiological implications of these
372 findings. We suggest that rather than simply being an indication of participants not meeting CHO
373 recommendations for performance, ketosis may be inevitable during certain ultra-endurance

374 events and that athletes will compete in a different physiological state than that which can be
375 assumed for races where external supplies are available. This has practical implications for the
376 self-sufficient, multi-stage athlete who must then manage the negative impacts of such an
377 extreme dietary and metabolic shift whilst competing in an already physiologically stressful
378 situation.

379
380 Although fat-oxidation rates have been shown to double following a ketogenic diet, this has not
381 translated into performance improvements for elite endurance athletes (Burke et al. 2017;
382 McSwiney et al. 2018). However, a benefit may exist for ultra-endurance athletes who are
383 working at lower intensities for much longer periods of time with low CHO availability (Burke
384 2015). Recent evidence also suggests that long-term ‘keto-adapted’ athletes (those regularly in a
385 ketogenic state for at least 6 months) not only have equivalent glycogen stores compared to
386 athletes on high CHO diets, but are also able to replenish these stores in the absence of dietary
387 CHO (Volek et al. 2016). This may be a crucial benefit for multi-stage athletes trying to recover
388 for subsequent days of racing and suggests that incorporating periods of ketosis into training
389 periods may better prepare athletes for these types of competitions. It is plausible that when
390 confronted with such large energy deficits, athletes with greater metabolic flexibility, and
391 specifically the ability to produce and utilise ketones more quickly would perform better. Our
392 results show a trend in this direction and since to the best of our knowledge there are no studies
393 quantifying the ‘efficiency’ of ketosis (how quickly people may become ketogenic without side
394 effects), this deserves further exploration in future research. These results also raise the question
395 of whether athletes might benefit from starting their events already in nutritional ketosis (without
396 energy restriction) to avoid the adaptation period while racing.

397

398 In the present study we report a strong relationship between CHO intake and performance.
399 Carbohydrate is undoubtedly ergogenic and we would expect to see increased CHO intake
400 improve performance in ultra-endurance events (Mahon et al. 2014; Stellingwerff and Cox 2014).
401 However, nutritional intakes in this study were constrained by food choices made by the
402 participants prior to starting the race. While evening meals were of similar composition across
403 the cohort (typically commercial freeze-dried meals) the faster competitors took a higher
404 proportion of 'sports nutrition' products (gels, bars) for daytime consumption which were higher
405 in CHO than 'real' foods taken by the slower competitors (meat jerky, nuts, seeds, cheese). It
406 therefore cannot be ruled out that CHO intake may have been coincidental to better performance
407 rather than causal. Since total weight of food is a key consideration, it is unclear as to whether
408 CHO or total energy should be prioritised when optimising race nutrition for a self-supported
409 event. The magnitude of the energy deficit induced by physical exercise in these events suggests
410 that increasing energy content using energy dense high fat foods may improve performance, but
411 the performance benefits of CHO are undeniable, as long as they are available.

412
413 There is little data on the relationship between increased pack weight through increased
414 nutritional supplies (and therefore intake) and performance in multi-stage races. A recent case-
415 study suggests the benefits of increased energy consumption outweigh the detriments associated
416 with an increase in pack weight (Alcock et al. 2018), although carrying enough food to meet
417 energy requirements resulted in a pack weight of 14 kg. This contrasts with the mean 7.6 kg
418 carried by the top 10 competitors in this race and is more than 2.5 times the weight of the pack
419 of the winning competitor. The current literature provides little incentive to increase weight
420 carried if athletes are already performing well with much lighter packs and concomitant reduced
421 energy consumption. In these circumstances, these findings should direct future research into

422 appropriate training and preparation to attenuate potential ketogenic adaptation issues during
423 races and optimise performance.

424
425 β -hydroxybutyrate levels were not different from baseline on day one, however, we observed a
426 significant 5.4 fold increase from baseline on day two ($0.25 \pm 0.08 \text{ mmol}\cdot\text{L}^{-1}$ to 1.35 ± 0.60
427 $\text{mmol}\cdot\text{L}^{-1}$, $p = 0.0003$), which rose to a 6.4 fold increase from baseline on day three (0.25 ± 0.08
428 $\text{mmol}\cdot\text{L}^{-1}$ to $1.6 \pm 0.57 \text{ mmol}\cdot\text{L}^{-1}$, $p < 0.0001$). All participants exhibited reduced KET on day
429 four, albeit still significantly higher than baseline, and KET increased again on days five and six.
430 The universal drop in KET on day four was unexpected and does not have a ready explanation
431 given that it was unrelated to macronutrient intakes or changes in physical activity. Although
432 speculative, the combined GLUC and KET pattern is indicative of the starvation response as
433 identified by Cahill (1976), the initial stages of which have distinct adaptation phases. Previously
434 consumed meals will provide fuel for up to eight hours. During the subsequent 24 - 48 hours,
435 liver glycogen is used to maintain glucose homeostasis and ketone production increases.
436 Thereafter, as liver glycogen is depleted, gluconeogenesis increases while ketone utilization
437 reduces the demand for glucose from tissues. This results in a temporary increase in blood
438 glucose and concomitant drop in ketone production. After four to five days, major adaptations to
439 energy metabolism occur and ketone utilization increases with a concurrent reduction in
440 gluconeogenesis (Cahill 1976). The starvation response has previously been identified in athletes
441 participating in a 1 230 km ultra-endurance cycling event (Geesmann et al. 2017). During the 54
442 hour event, Geesmann et al. (2017) found that an energy deficit of $23.2 \pm 19.1 \text{ MJ}$ resulted in the
443 suppression of testosterone, leptin and IGF-1. In some athletes these remained suppressed for up
444 to three days despite *ad-libitum* intake during recovery. Given the large energy deficits
445 accumulated over five days by participants in this study, further research into the effects of
446 starvation on the metabolic and hormonal health of ultra-endurance athletes is warranted. This is

447 of particular concern for athletes who train for, and compete in, several self-sufficient multi-
448 stage ultra-endurance events per year.

449
450 While a major strength of this study was its applied nature, contributing to understanding
451 metabolic shifts in athletes in a real-world, competitive environment, limitations must be
452 considered. Energy expenditure was not directly quantified but the estimated energy
453 expenditure of $2.3 \text{ MJ}\cdot\text{hr}^{-1}$ whilst racing is in line with previous studies on ultra-endurance racing.
454 These include a 24 hr trail race ($2.3 \text{ MJ}\cdot\text{hr}^{-1}$) (Costa et al. 2014), a 24 hr lab-simulated adventure
455 race ($3.1 \text{ MJ}\cdot\text{hr}^{-1}$), and a 6 day adventure race ($2.1 \text{ MJ}\cdot\text{hr}^{-1}$) (Enqvist et al. 2010). The average
456 daily energy expenditure in our study ($22.7 \text{ MJ}\cdot\text{day}^{-1}$) was larger than previously reported for a
457 multi-day ultramarathon of a similar format (5 days, 225 km, 16.0 to $20.0 \text{ MJ}\cdot\text{day}^{-1}$) (Costa et al.
458 2013) although in the study of Costa et al. (2013) overall distance and running time was less than
459 our study. Additionally, participants in the study of Costa et al. (2013) had their food and
460 equipment transported each day meaning that they could take, and thus consume, more food
461 (intake in our study: $9.6 \pm 2.6 \text{ MJ}\cdot\text{day}^{-1}$ compared to Costa et al. 2013: $14.0 \pm 3.1 \text{ MJ}\cdot\text{day}^{-1}$).
462 Furthermore, this daily transportation of provisions also means load carriage was reduced,
463 potentially resulting in a lower energy expenditure (Lucas et al. 2016), given that the cohort in
464 the present study had a mean starting pack weight of 8.6 kg. Therefore, we believe estimates of
465 energy expenditure and subsequent deficits in the present study are reasonable.

466
467 Due to logistical constraints in the field, morning pre-stage data collection of blood substrates
468 was not possible but would have added a greater understanding of changes over the course of the
469 race. Furthermore, although capillary testing is a well-recognised method of measuring ketones
470 (Brewster et al. 2017), venous blood samples could take into account changes in blood plasma
471 volume as well as identifying other biomarkers. It has been shown that blood plasma volume

472 increases over the course of a multi-stage ultramarathon (Alcock et al. 2018; Costa et al. 2014)
473 suggesting that the participants' ketone levels may have been even higher than recorded.
474 However, given the remote nature of this race the logistics of storing, transporting and analysing
475 whole blood was beyond the scope of the study.

476

477 ***Conclusion***

478 Health and performance related issues in ultra-endurance athletes have been well established and
479 the focus is currently on solving these issues (e.g. gut-training for optimal CHO intake). Unlike
480 races where optimal nutritional strategies may be applied through external access to food self-
481 sufficient multi-stage ultra-marathons restrict intake to the load the athlete is prepared to carry
482 from day one.. This is the first study to document changes in blood glucose and β -HB
483 concentrations and concomitant nutritional intakes during a self-sufficient multi-stage ultra-
484 marathon. We showed that all participants were ketogenic by day two. This suggests that rather
485 than being a nutritional choice, competing in a state of ketosis may be unavoidable in multi-stage
486 events where load carriage considerations encourage energy and CHO restriction. .Given the
487 potential negative impacts associated with such an extreme metabolic shift in athletes
488 unaccustomed to such restriction (fatigue, increased perceived effort and changes to the
489 hormonal milieu), prior keto-adaptation could be a useful strategy to improve health and
490 performance in these athletes, however further work is required to elucidate the benefits of such
491 an approach.

492

493 ***Acknowledgements***

494 The authors wish to thank the all the participants whose willingness to participate and generosity
495 of spirit in staying with it all until the end is greatly, and gratefully, appreciated. Thanks also go

496 to the management team at Racing the Planet for their enthusiasm for the study and their
497 willingness in allowing the research to take place.

498

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Table 1: Participant characteristics

	<i>Participant Characteristics (n= 13, 8 males, 5 females)</i>		
	Pre-race	Day 6	Cohen's <i>d</i>
Age (years)	40 ± 8	-	
Height (cm)	175.1 ± 8.1	-	
Body mass (kg)	73.1 ± 11.8	70.6 ± 11.6 *	0.1
Sum of 4 skinfolds (mm)	38.1 ± 12.2	32.0 ± 10.8 *	0.3
Starting pack weight (kg)	8.6 ± 1.3	-	

Note: Mean ± SD; * $p < 0.001$ vs pre-race;

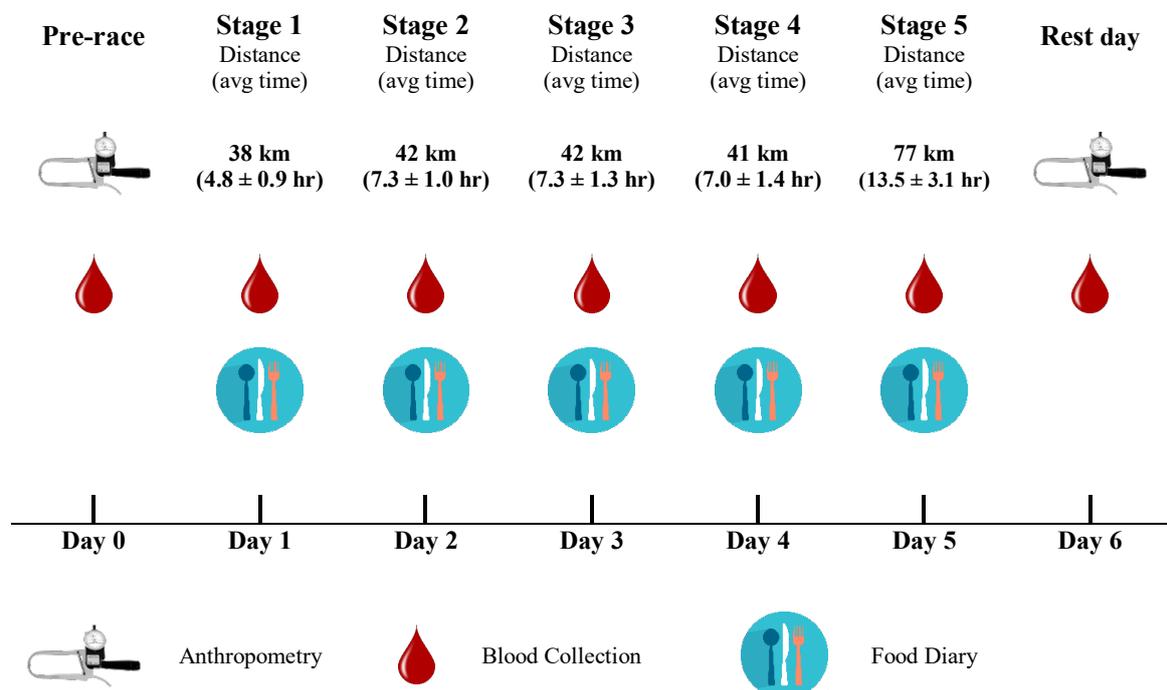


Figure 1. Schematic of study protocol. Blood samples were taken immediately post-stage every day at the finish line

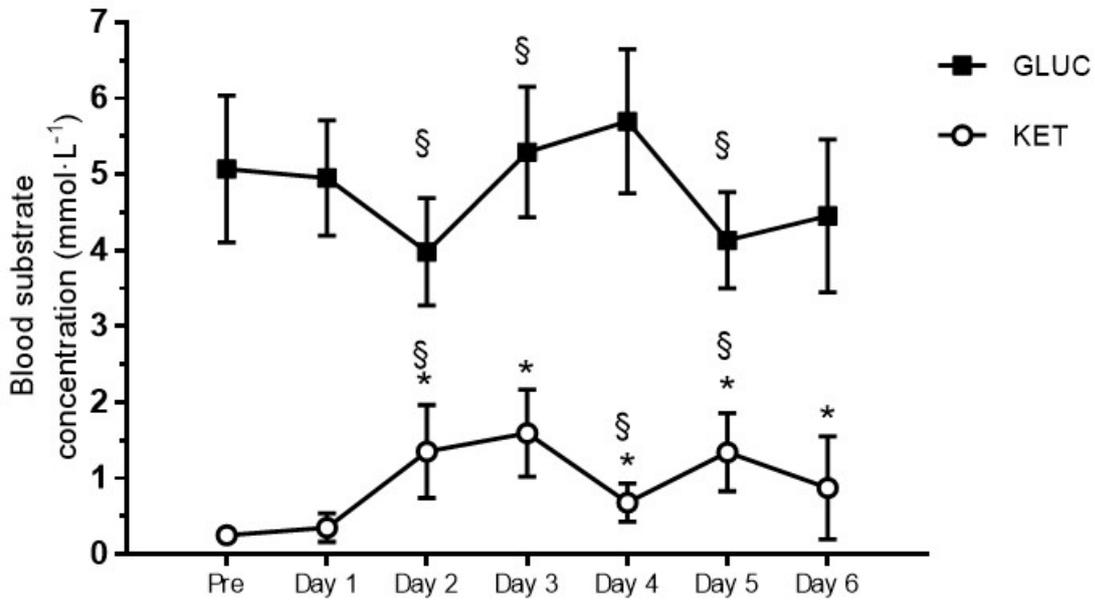


Figure 3. Blood GLUC and KET concentrations. * denotes a significant change from baseline. § denotes a significant change from the day before. Data are presented as mean \pm SD.

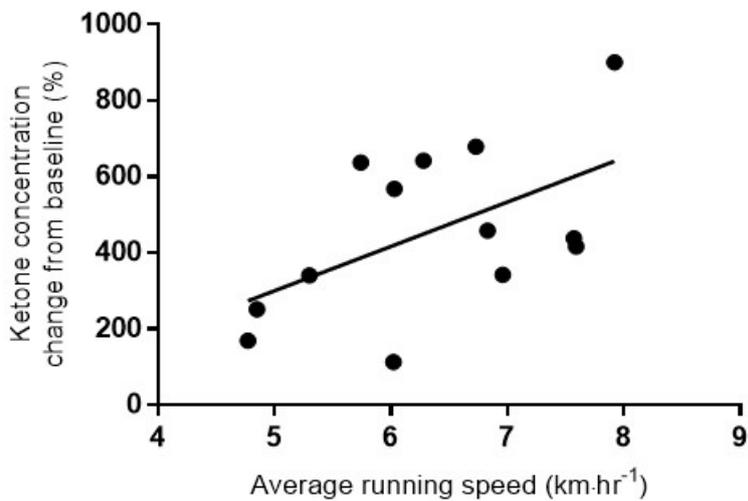


Figure 4. The relationship between the change in ketone concentration from baseline on day two and overall performance as defined by average speed throughout the race ($r = 0.54$, $p = 0.06$)